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(57) Abstract

A pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, is provided. The agent comprises a short chain fatty acid, or a pharmaceutically acceptable salts, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal. Preferably the agent comprises calcium acetate in a shellac coating.

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Anti-Atherosclerotic and Anti-Thrombotic Agent and the Use Thereof

INTRODUCTION AND BACKGROUND TO THE INVENTION

This invention relates to a pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity and the use thereof.

It is generally known that atherosclerosis is primarily caused by increased levels of cholesterol in human beings and that thrombosis is caused by the polymerisation of fibrin to form fibrin clots.

Low density lipoprotein cholesterol (LDL-C), occurring in relatively high concentrations, is particularly responsible for an increase in cardiovascular disease, especially when the LDL-C is oxidised by free radicals such as lipid peroxides. Although it is has been reported that dietary fibre can modify lipid metabolism in man, no effects of fibre, fibre components or metabolites thereof on lipid peroxidation have been reported.

It is further known that fermentable non-starch polysaccarides such as pectin, are fermented in the colon of a mammal to short chain fatty acids or derivatives thereof, such as acetate, propionate and butyrate. The butyrate is absorbed by the colon cells while the propionate and acetate

move to the liver. The propionate is retained in the liver while the acetate is distributed throughout the cells and plasma of the mammal.

OBJECT OF THE INVENTION

It is an object of the present invention to provide a novel pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity and the use thereof.

SUMMARY OF THE INVENTION

According to the invention a pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, is provided which comprises a short chain fatty acid, or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

Preferably the pharmaceutically acceptable salt of the short chain fatty acid is the calcium salt thereof.

Preferably the short chain fatty acid comprises acetic acid.

The protective coating may comprise a natural or synthetic resin such as shellac.

The pharmaceutical agent preferably comprises calcium acetate in the form of a capsule, tablet or pill coated with such a resin.

Preferably the agent comprises between 0,1 grams and 100,0 grams of the acetate.

According to another aspect of the invention a method for the treatment or prevention of any one or more of said conditions in a mammal includes the step of administering to the colon of a mammal an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof.

Preferably the agent is administered to the colon, via the digestive track of the mammal.

According to another aspect of the invention there is provided the use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof in a method for the treatment or prevention of any one or more of said conditions in mammals.

According to another aspect of the invention there is provided the use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in the manufacture of a medicament for use in a method for the treatment or prevention of any one or more of said conditions in mammals.

Further according to the invention, the aforesaid method includes the step of administering the agent orally in the form of a capsule, pill or tablet coated with a protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but soluble or digestible in the colon of said mammal.

Still further according to the invention the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

Still further according to the invention the short chain fatty acid is acetic acid.

Still further according to the invention the protective coating comprises a natural or synthetic resin such as shellac.

Applicant has found that the aforesaid clinical effects can be attained by administering the agent to a human being in an amount of between 0,1 gram and 100,0 gram at least once a day.

SPECIFIC DESCRIPTION OF THE INVENTION

The invention will now be described further by way of the following non-limiting examples.

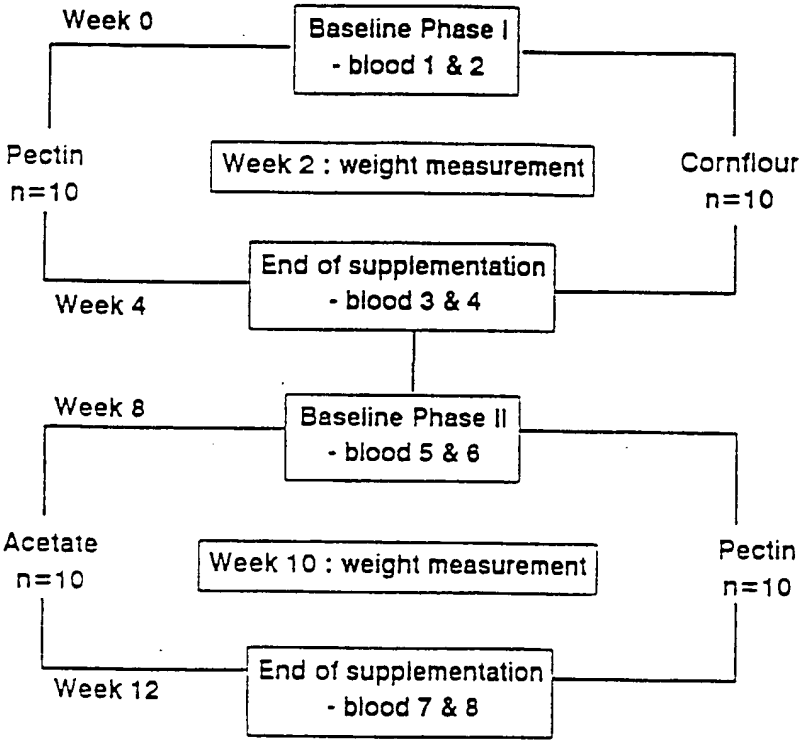
The codes used in the examples denote the following:

ApoA	--	APO-PROTEIN A
ApoB	--	APO-PROTEIN B
BMI	--	BODY MASS INDEX = WEIGHT/(LENGTH)^2
DBP	--	DIASTOLIC BLOOD PRESSURE
FFA	--	FREE FATTY ACIDS
FFA/ALB	--	FREE FATTY ACID TO ALBUMIN RATIO
HAEMATOCRIT	--	% PACKED CELLS IN BLOOD
HDL-C	--	HIGH DENSITY LIPOPROTEIN CHOLESTEROL
IR	--	INSULIN RESISTANCE
LDL-C	--	LOW DENSITY LIPOPROTEIN CHOLESTEROL

LP(a)	--	LIPOPROTEIN (a)
MPC	--	MACROMOLUCULAR PROTEIN COMPLEX
SBP	--	SYSTOLIC BLOOD PRESSURE
TBARM	--	THIOBARBITURIC REACTIVE SUBSTANCES OF MALONDEALDEHYDE
TC	--	TOTAL CHOLESTEROL
TG	--	TRIGLYCERIDES
TP	--	TOTAL PROTEIN
μT	--	MASS LENGTH RATIO FROM TURBIDITY

EXAMPLE 1

The respective effects of pectin and an acetate when administered to the colon of a mammal were determined during a first experiment. The experiment was conducted in the following two phases:



Twenty human males participated in the experimentation and these subjects were not on any medication for any chronic diseases at the time, and also had no history of cardiovascular disease. All the subjects were at the time following a relatively high fibre, low fat diet. During the first phase ten subjects consumed a total of 15 grams of pectin per day in four aliquots, while the other ten consumed a total of 15 grams of placebo (starch) per day in four aliquots.

During the second phase, the first group consumed a total of 7,5 grams of calcium acetate per day in four aliquots and the second group consumed a total of 15 grams of pectin per day in four aliquots. The calcium acetate was administered in capsules which were coated with a protective coating comprising a resin known commercially as shellac. This protective coating is resistant to digestion and solution in the stomach and small intestines, but not resistant to the enzymes of the organisms usually found in the colon, so that the calcium acetate was thus released in the colon. Details of the subjects are given in Table 1.

TABLE 1: PERSONAL DETAILS OF SUBJECTS PARTICIPATING IN THE EXPERIMENTATION

VARIABLE	PECTIN : PHASE 1 ACETATE : PHASE 2	PLACEBO : PHASE 1 PECTIN : PHASE 2
SEX	Male	Male
AGE (years)	45.27 ± 12.24	42.0 ± 10.22
SBP (mmHg)	125.9 ± 9.7	125.0 ± 14.3
DBP (mmHg)	81.3 ± 9.77	79.5 ± 10.1
Activity level	Medium	Medium
Cardiovascular events	No history	No history
WEIGHT (kg)	89.50 ± 11.81	92.10 ± 15.03
BMI (kg^1m^{-2})	27.50 ± 2.99	29.70 ± 3.09
MEDICATION	None	None

Blood samples where taken from the subjects after each phase and a large number of variables where tested. The results of these tests are given in Tables 2 to 5.

Table 2 Means and standard deviations of body weight and BMI changes

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
BODY WEIGHT (kg)	89,50 ± 11,81	89,10 ± 11,92	92,10 ± 15,03	92,10 ± 15,54	92,07 ± 15,54	91,55 ± 14,55	88,16 ± 12,35	83,04 ± 10,80
BMI (kg/m^2)	27,50 ± 2,99	27,40 ± 2,98	29,70 ± 3,09	29,50 ± 3,04	29,46 ± 3,03	29,32 ± 2,82	26,90 ± 2,82	25,65 ± 2,62

Table 3 Means and standard deviations of baseline and end of supplementation haemorhe logical and haemostatic variables

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
HAEMATOCRIT (%)	48,70 ± 2,45	48,10 ± 2,69	48,70 ± 2,45	48,10 ± 2,64	47,38 ± 2,67	45,39 ± 2,55	48,55 ± 1,88	46,83* ± 1,85
HAEMOGLOBIN (mmol/l)	10,30 ± 1,09	9,60* ± 0,98	10,30 ± 0,91	10,60 ± 0,98	10,82 ± 1,07	9,75* ± 0,84	11,12 ± 0,34	10,48 ± 0,50
VISCOSITY (cP)	1,81 ± 0,08	1,60* ± 0,19	1,80 ± 0,09	1,70* ± 0,07	1,75 ± 0,10	1,62* ± 0,13	1,92 ± 0,22	1,61* ± 0,22
COMPACTION (%)	21,51 ± 3,65	30,16* ± 4,41	21,60 ± 3,85	24,63 ± 3,47	20,67 ± 5,86	31,53* ± 6,09	22,47 ± 2,90	32,21* ± 9,15
μ_1 (Dal/cm x 10^{13})	19,94 ± 6,27	24,80* ± 4,22	19,80 ± 5,96	19,10 ± 10,49	19,02 ± 11,93	32,10* ± 7,52	22,93 ± 10,41	34,28* ± 5,42
VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
PERMEABILITY (x 10^{11}cm²)	279,58 ± 101,16	336,25* ± 119,06	275,5 ± 116,4	307,09 ± 72,98	131,18 ± 99,94	285,36* ± 84,50	212,52 ± 76,32	306,81* ± 80,83
LYSIS TIME (t50%)	285,6 ± 16,13	232,9* ± 17,9	205,5 ± 14,9	221,4 ± 10,9	285,6 ± 16,13	132,9* ± 17,9	251,9 ± 10,7	130,3* ± 14,8
MPC (g/l)	0,1218 ± 0,0394	0,0836* ± 0,0395	0,109 ± 0,083	0,097 ± 0,059	0,1002 ± 0,029	0,0807* ± 0,0314	0,1146 ± 0,0429	0,0852** ± 0,0371
CLOT (FIBRIN) (g/l)	2,22 ± 0,47	1,90* ± 0,37	2,30 ± 0,44	2,10 ± 0,33	2,55 ± 0,70	1,86* ± 0,37	2,00 ± 0,28	1,62* ± 0,16
FIBRINOGEN (g/l)	3,51 ± 0,62	3,30 ± 0,48	3,60 ± 0,62	3,62 ± 0,35	4,11 ± 0,90	3,72 ± 0,62	4,10 ± 1,44	3,64 ± 0,91

Table 4 Means and standard deviations of baseline and end of supplementation lipid variables

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
TC (mmol/l)	6,50 ± 0,27	5,67* ± 0,48	6,60 ± 0,97	6,40 ± 0,79	6,89 ± 0,86	6,07 ± 0,79	6,55 ± 0,63	5,81* ± 0,49
LDL-C (mmol/l)	4,70 ± 0,35	4,10* ± 0,59	4,80 ± 0,98	4,60 ± 0,63	5,17 ± 0,60	4,59 ± 0,69	4,97* ± 0,53	4,20* ± 0,38
HDL-C (mmol/l)	1,20 ± 0,18	1,03* ± 0,14	1,20 ± 0,19	1,10 ± 0,26	0,92 ± 0,01	1,13* ± 0,27	1,11 ± 0,14	1,18 ± 0,11
% HDL-C (%)	18,30 ± 3,07	18,20* ± 2,64	17,70 ± 2,29	17,30 ± 3,40	15,46 ± 0,01	18,79* ± 4,48	17,04 ± 0,69	20,32* ± 2,98
H_2O_2 (μM)	1,70 ± 0,76	0,84* ± 0,38	1,50 ± 0,52	1,45 ± 0,78	1,20 ± 0,33	0,73* ± 0,23	1,27 ± 0,48	0,81* ± 0,22
VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
ApoA (mmol/l)	1,60 ± 0,14	1,23* ± 0,12	1,50 ± 0,18	1,40* ± 0,22	1,53 ± 0,18	1,39* ± 0,22	1,50 ± 0,16	1,40* ± 0,15
ApoB (mmol/l)	1,70 ± 0,15	1,29* ± 0,12	1,70 ± 0,28	1,50* ± 0,18	1,77 ± 0,65	1,39* ± 0,16	1,47 ± 0,15	1,34* ± 0,14
TG (mmol/l)	2,00 ± 0,84	1,78 ± 0,64	2,10 ± 0,98	2,00 ± 0,64	1,99 ± 0,59	1,78 ± 0,39	0,65 ± 0,59	1,33* ± 0,33
LP(a) (mmol/l)	349,23 ± 317,37	251,93* ± 213,27	281,0 ± 142,08	249,33 ± 129,33				
TBARM (μM)	0,60 ± 0,59	0,30 ± 0,20	0,50 ± 0,25	0,90 ± 0,84	2,06 ± 1,52	0,61* ± 0,53	1,47 ± 0,64	1,07 ± 0,83

Table 5 Means and standard deviations of baseline and end of supplementation metabolic variables

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
ACETATE (µmol/l)	50,65 ± 28,49	90,52* ± 43,14	44,20 ± 17,07	42,96 ± 21,55	37,81 ± 9,52	67,97* ± 25,95	37,63 ± 16,31	54,31* ± 12,12
FFA (mmol/l)	0,39 ± 0,03	0,31* ± 0,02	0,33 ± 0,01	0,43* ± 0,09	0,48 ± 0,06	0,40* ± 0,03	0,59 ± 0,05	0,45* ± 0,04
TP (g/l)	68,55 ± 7,99	66,84 ± 6,33	65,51 ± 5,26	64,09 ± 6,33	72,36 ± 4,04	75,21 ± 6,84	71,69 ± 4,52	71,52 ± 3,26
ALBUMIN (g/l)	47,23 ± 8,31	47,79 ± 2,96	47,46 ± 1,63	45,36 ± 6,10	45,53 ± 5,35	45,48* ± 2,69	43,09 ± 3,16	45,50 ± 2,12
VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
INSULIN (µU/ml)	10,95 ± 6,85	11,25 ± 6,34	17,14 ± 12,17	17,98 ± 13,21	18,55 ± 13,16	13,83 ± 7,34	8,96 ± 5,19	7,61 ± 4,78
GLUCOSE (mmol/l)	3,98 ± 0,34	3,72 ± 0,38	3,99 ± 0,59	3,96 ± 0,64	4,29 ± 1,52	4,00 ± 0,61	3,58 ± 0,39	3,78 ± 0,34
IR	4,29 ± 2,70	4,16 ± 2,38	7,16 ± 5,94	7,46 ± 6,02	9,40 ± 5,00	5,77 ± 3,77	3,22 ± 1,87	2,93 ± 1,93
FFA/ALB (X 10')	8,26 ± 0,43	6,49* ± 0,59	6,95 ± 0,27	9,48* ± 0,80	10,50 ± 0,44	8,78* ± 0,63	13,69 ± 0,88	9,89* ± 0,50

The results of the above experiments will now be discussed briefly.

BODY WEIGHT AND BODY MASS INDEX (BMI) CHANGES

As is evident form Table 2, no significant changes in body weight or BMI were observed in any of the groups during phase 1. The acetate supplement (phase 2), however, caused a decrease (from 88.16 ± 12.35 kg to 83.09 ± 10.80 kg) in body weight. Although this decrease may not be of statistical significance, it can be clinically significant in the cases of those subjects who lost weight.

HAEMORHEOLOGICAL AND HAEMOSTATIC VARIABLES

As is evident from Table 3, pectin supplementation for both groups during both phases caused a significant decrease in the clot lysis time, Macromolucular Protein Complex (MPC), clot fibrin content, Haemoglobin (Hb), plasma viscosity, and a significant increase in fibrin clot compaction, mass length ratio from turbidity (μT) and clot permeability.

Except for a significant decrease in the plasma viscosity in the placebo group during phase 1 (from 1.80 ± 0.09 to 1.70 ± 0.07 cP), no other changes were observed in this group.

It is furthermore clear from Table 3 that acetate supplementation caused a significant decrease in Haematocrit (Ht), Hb, plasma viscosity, MPC, clot fibrin content and clot lysis time, while significant increases were measured in clot compaction and permeability. Although the change in fibrinogen was not significant, it is worthy to note that acetate supplementation caused a 11.2 % decrease in the total plasma fibrinogen concentration of the group.

LIPID CHANGES

As appears from Table 4, pectin supplementation caused significant decreases in total cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C), and Apoprotein A (ApoA), Apoprotein B (ApoB), Lipoprotein (a) (Lp(a)), Tribarbituric Reactive Substances of Malondealdehyde (TBARM) and in hydrogen peroxide (H_2O_2) during phase 1. HDL-C was significantly increased during phase 2.

It is also apparent that ApoA decreased substantially in the placebo group. A significant decrease in ApoB was also measured. No other changes were significant.

It therefore appears that acetate supplementation caused a substantial decrease in TC, ApoA, ApoB, TG, and H_2O_2, while a significant increase in the %HDL-C was also evident.

METABOLIC VARIABLES

As appears from Table 5, which reflects the mean (SD) changes in some metabolic variables of both groups during both phases, pectin supplementation caused a significant increase in acetate levels and a significant decrease in Free Fatty Acid (FFA) levels and ratio of FFA/albumin.

Except for a significant increase in the ration of FFA/albumin, no other significant changes were found in the placebo group.

It is also clear that acetate supplementation caused a substantial increase in acetate levels, and a significant decrease in FFA and ratio of FFA/albumin.

EXAMPLE 2

The effect of the acetate on the fibrin clot structure was further determined by in vitro studies and the results and a discussion thereof are given below.

ACETATE AND FIBRIN CLOT STRUCTURE

The effect of different concentrations of acetate on fibrin clot structure properties (n = 5 each variable tested), is reflected in Table 6.

Table 6 The effect of different concentrations of acetate on fibrin clot structure properties (n=5 for each variable tested)

[Acetate] (μmol/L)	Permeability (x 10^{11}cm^2)	μT (daltons/cm x 10^{12})
0	90.67 ± 8.00	14.92 ± 0.15
75	110.4 ± 5.17*	17.44 ± 0.20*
100	118.0 ± 6.03*	17.95 ± 0.22*
150	134.0 ± 5.02*	19.51 ± 0.17*

* differ significantly from 0 μmol/L acetate ($p < 0.05$; Student t-test)

It is evident from Table 6 that as the acetate concentration increased progressively from 0 μmol/L to 75, 100 and 150 μmol/L, the permeability increased accordingly. Fibre thickness from turbidity (μT) increased significantly. The clot lysis time decreased substantially, indicating enhanced fibrinolysis with progressive acetate concentrations. These

changes in network characteristics do not arise from altered fibrinogen conversion because fibrin content did not alter substantially in the concentration range of the acetate tested. These findings probably indicate that the fibrin in the presence of acetate shows increased lateral polymerization. Therefore a greater amount of fibrin is incorporated into the major network and the cross linking in the network is different to that of the control network.

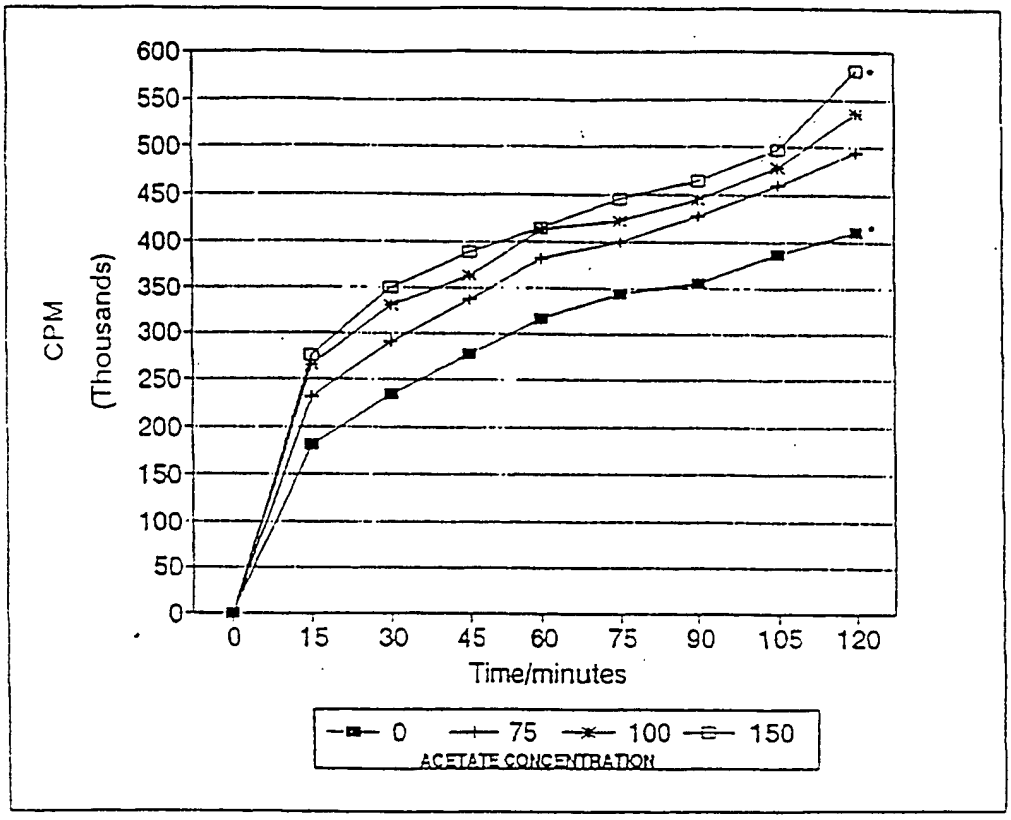
The effect of different concentrations of acetate on clot fibrin content and sample viscosity (n = 5 for each variable tested) is reflected in Table 7 and the relation between fibrin network lysis and acetate concentrations is depicted in Figure 1.

Table 7 The effect of different concentrations of acetate on clot fibrin content and sample viscosity (n=5 for each variable tested)

[Acetate] (μmol/L)	Clot [FIBRIN] (g/L)	Lysis time (t½ / minutes)
0	1.35 ± 0.05	148.50 ± 2.50
75	1.36 ± 0.03	140.25 ± 2.23 *
100	1.37 ± 0.07	129.15 ± 1.66 *
150	1.39 ± 0.05	123.29 ± 2.02 *

* differ significantly from 0 μmol/L acetate ($p < 0.05$; Student t-test)

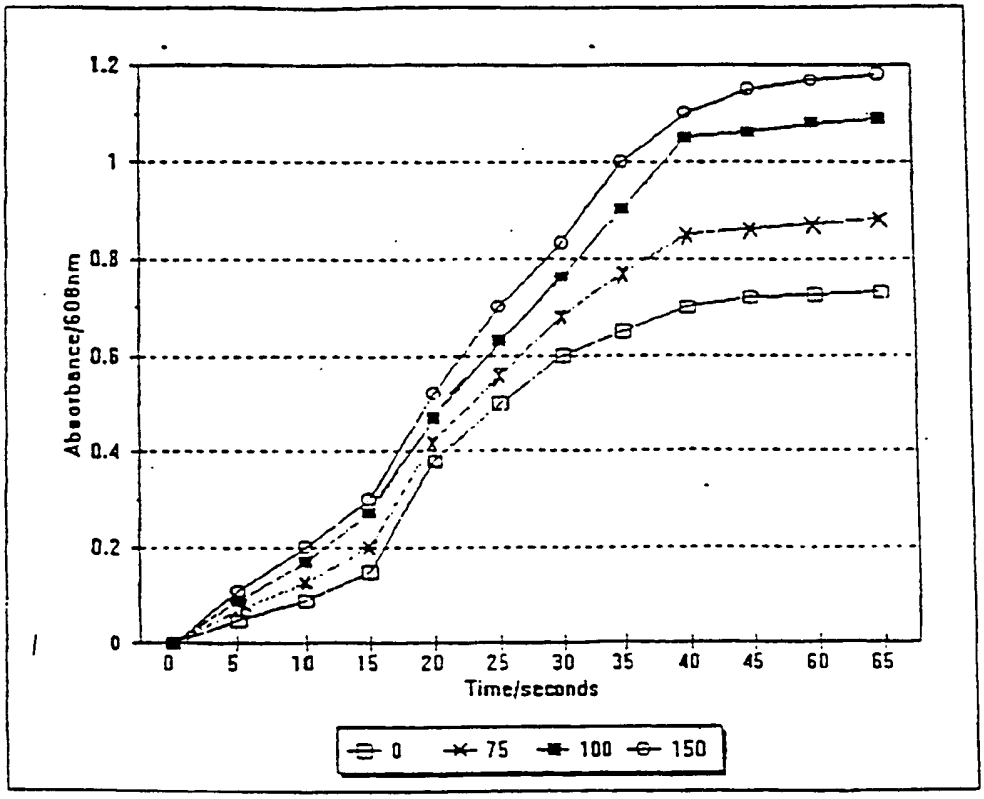
Figure 1: Lysis by streptokinase of fibrin networks developed with different concentrations acetate (n = 5 for each concentration tested)



Referring to Table 7 and Figure 1, the lysis rate of radioactive-labelled fibrin clots in the presence of different concentrations of acetate were quantified by measuring released I^{125} in the medium over a determined time period. It therefore appears that progressive acetate concentrations enhanced fibrinolysis.

Referring to Figure 2, the kinetics of network growth were subsequently investigated by continuously recording changes in turbidity at 608 nm, during network development under identical experimental conditions. As depicted in Figure 2, progressive increase in acetate enhanced the entire kinetics. The lag phase became shorter, the increase in turbidity was faster and the equilibrium turbidity was proportionally increased.

Figure 2: Turbidity curve of fibrin formation in the presence of different acetate concentrations



ACETATE AND LIPID PEROXIDATION

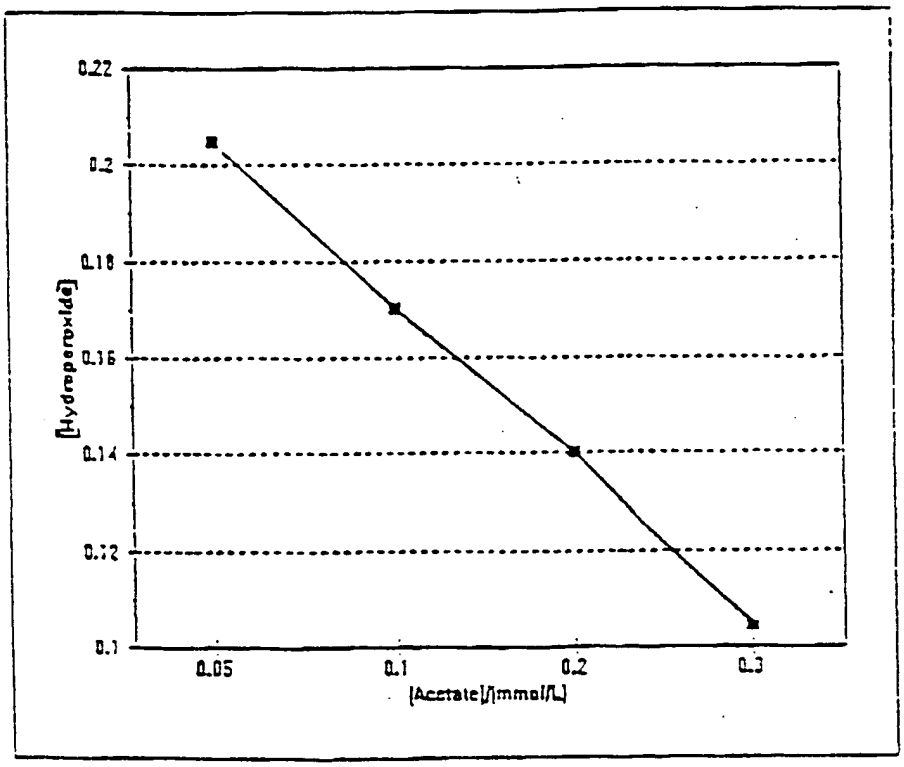
The effect of acetate on peroxidation of blood lipids *in vitro* (n = 5 for each measurement) is reflected in Table 8 and the relationship between the inhibition of peroxidation and acetate concentration is depicted in figure 3.

Table 8 The effect of acetate on peroxidation of blood lipids *in vitro* (n=5 for each measurement)

[Acetate] (μM)	[Hydroperoxide] x 10^{-6} M	% Inhibition
0.00 mmol/L	8.41 ± 0.20	0
0.05 mmol/L	4.46 ± 0.15 *	46.97
0.10 mmol/L	3.70 ± 0.22 *	56.01
0.20 mmol/L	3.04 ± 0.23 *	67.11
0.30 mmol/L	2.26 ± 0.16 *	73.12

* differ significantly from 0 μmol/L acetate ($p < 0.05$; Student t-test)

Figure 3: The relationship between inhibition of peroxidation and acetate concentration *in vitro*



From Table 8 and Figure 3 it appears that there exists a linear analogy between the extent of free radical inhibition and acetate concentration. A 46.97, 56.01, 67.11 and 73.12 % inhibition of free radical formation was caused by 50 μM, 100 μM, 200 μM and 300 μM of acetate, respectively. All these changes were significant ($p < 0.05$). However, the graph of Figure 3 suggests that acetate does not inhibit peroxidation in full. From linear regression analysis, it seems that minium inhibition may cause a 56.12 % decrease of peroxidation *in vitro* ($r = 0.98$; $m = -0.836$). The

results showed that pectin supplementation caused a 49 % decrease in free radical content, which corresponds to an acetate concentration of 70 μM, if related to this *in vitro* study. This value is within physiological range. It is however, important to realize that the Cu^{2+} concentration used to induce oxidation, is a drastic measurement, causing spuriously high rates of oxidation.

NOVEL EFFECTS

Pectin supplementation caused no substantial changes in plasma fibrinogen levels. However, significant differences were found in the characteristics of networks developed in plasma of the pectin group. Networks were more permeable and had lower tensile strength. Their fibrin content decreased markedly. A decrease in fibrin content partially explains some of the altered network characteristics due to altered fibrin(ogen) conversion. These findings indicate that lateral polymerization was enhanced and a greater amount of fibrin was thus incorporated into the major fibre network. The increased major network fibre diameter is reflected in the turbidimetric measurement as shown in Figure 2. Fibrin fibre thickness seems to be determined by kinetics of its growth and differences in fibre diameter have been attributed to the kinetics of fibrin(ogen) breakdown and subsequently fibrin fibre assembly. It is known that mass-length ratio of fibrin fibre is determined by the rates of generation of the fibrin monomer and that of its

assembly into fibrin fibre. When thrombin is added to fibrinogen, the fibrin monomer is generated according to the relative amounts of enzyme and substrate.

Turbidimetric changes represented by the lag phase, phase of increasing turbidity and the equilibrium phase, collectively represent the breakdown of fibrinogen to fibrin monomer; the initial aggregation of monomer to protofibrils; and the growth of protofibrils to an opaque network. The lag phase corresponds to the time required for the overall action of thrombin on fibrinogen until the appearance of turbidimetrically detectable fibrin and includes the enzymatic breakdown of fibrinogen and the initial aggregation to protofibrils. The fibrinogen solution forms a gel during the early part of the second phase during which turbidity rises rapidly. The resulting increased thickness of fibres decreases the total contour length of the fibres thus increasing the permeability. Networks with fibres of increased thickness and permeability are less resistant to lysis. Increased clot compaction also denotes a decrease in the tensile strength of fibrin. Increase in permeability and decrease in tensile strength indicates a smaller degree of cross linkage of fibres within the network.

The changes in fibrin network characteristic (μT and clot lysis time) were directly associated with the changes in plasma acetate levels.

Acetate supplementation did not cause a significant change in plasma fibrinogen levels, but a tendency of an 11.2 % decrease was observed in this group. Significant differences were also found in the characteristics of fibrin networks developed in plasma. These results were also observed with the results of the pectin group. Changes in clot structure properties were also associated with the changes in acetate levels. These results strongly suggest that the effect of pectin on clot structure characteristics were mediated by acetate.

Progressive amounts of acetate were used *in vitro* to investigate the possibility that acetate may directly be responsible for changes of fibrin clot structure characteristics *in vivo*, and rule out the effect of other possible changes that occurred in the plasma medium. The results indicated that acetate directly influence fibrin clot structure properties in the same manner as during pectin and acetate supplementation. Increasing amounts of acetate caused significant changes in the clot characteristics.

Although it is known that dietary fibre can modify lipid metabolism in man, no effects of fibre or fibre components or metabolites on lipid peroxidation have previously been reported. During the experiments, pectin supplementation caused a significant decrease of 49% in the hydrogen peroxide content of blood lipids. This effect was concomitant with a

decrease in total cholesterol. The change in lipid peroxides was directly associated with the change in TC and acetate levels.

Acetate supplementation caused a significant decrease in the free radical content of blood lipids. This effect was concomitant with a decrease in total cholesterol. The change in free radical concentration was directly associated with the change in TC and acetate levels.

The direct effect of acetate on lipid peroxidation was performed *in vitro* to rule out the effect of significant decreases in TC as reported for the acetate and pectin intervention results. The results showed that progressive amounts of acetate *in vitro* decreases the susceptibility of lipoproteins against free radical attack.

A clinically significant, but statistically insignificant decrease in body weight of 5.07 kg of the acetate supplemented subject group was observed. It was previously showed that acetate inhibits food intake in sheep. The acetate effect can therefore possibly be ascribed to be through direct mechanisms and a decrease in food intake. No weight reduction were measured in the pectin supplemented subject group. The weight loss with acetate supplementation probably contributed to the lowering of TC and TG.

POSSIBLE MECHANISMS

The results showed that both acetate and pectin *in vivo* induce alterations in network characteristics. However, pectin and acetate *in vivo* also showed significant effects on some other metabolic variables. Plasma is an aqueous mixture of proteins, lipids, carbohydrates, amino acids, salts and other substances. A change in any of these constituents of plasma would directly be reflected in the characteristics of fibrin networks. It would therefore seem that acetate and pectin can modify network characteristics by a combination of its effect on metabolism (modulating mechanism), possible direct effects (steric exclusion, etc.), and altered fibrin conversion (kinetic mechanism).

The mechanism underlying these differences is not clear at present, but in the investigation with artificially added acetate the reagents were added only a few minutes before developing the network. The changes induced are thus from a direct effect of acetate on fibrin. Therefore it appears that in the presence of acetate added in this fashion, the networks developed simulated changes observed in network characteristics of both acetate and pectin supplemented subject plasma. This indicates that acetate may directly be responsible for partial changes in fibrin network characteristics.

The physiochemical nature of acetate defines the behaviour of this acid in living organisms. Molecules (such as acetate) of compounds contain O-H groups are attracted to each other by intermolecular force caused by the difference in the electronegativity of oxygen and hydrogen atoms. This gives acetate the ability to form hydrogen bonds between O-H, H-F, H-Cl and H-N. Hydrogen bonding is the key factor determining the characteristics of acetate in solution. There are two types of hydrogen bonding, intramolecular and intermolecular. Intermolecular bonding may be a link to the effects of acetate on fibrin clot structure *in vitro* and *in vivo*. Fibrinogen is a very large molecule with an array of different bonds. It is not impossible for acetate to form hydrogen bonds with the fibrinogen molecule, having both O-H and H-N groups. This may have steric effects on the fibrinogen molecule, causing a change in fibrinogen-thrombin interaction, which will consequently lead to an altered clotting process. This should lead to alterations in fibrin clot structure.

Both pectin and acetate decreases peroxidation of blood lipids *in vivo*. Excluding acetate, no other measured variable could explain this anti-oxidative effect of pectin and acetate *in vivo*. The underlying mechanism is not clear. From the *in vitro* results it seems that acetate inhibits lipid peroxidation directly. This indicates that pectin fermentation produces substances (acetate) with anti-oxidant properties. This may be direct

evidence that acetate protects against lipid peroxidation by inhibiting the release of free radicals, rather than protecting the blood lipids against them.

It will be appreciated that short chain fatty acids, such as acetic acid, or pharmaceutically acceptable salts, derivatives or precursors thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestines of a mammal, but soluble and digestible in the colon of such mammal, could be used as a pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity and the use thereof. It will be appreciated further that such short chain fatty acids can further be used in methods for the treatment or prevention of any one or more of said conditions in mammals.

It will be appreciated still further that there are no doubt a large number of variations in detail possible with the invention as hereinbefore described without departing from the scope and/or spirit of the appended claims.

CLAIMS

1. A pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, comprising a short chain fatty acid, or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

2. A pharmaceutical agent according to claim 1 wherein the pharmaceutically acceptable salt of the short chain fatty acid is the calcium salt thereof.

3. A pharmaceutical agent according to claim 1 or claim 2 wherein the short chain fatty acid comprises acetic acid.

4. A pharmaceutical agent according to any one of the preceding claims wherein the protective coating comprises a natural or synthetic resin such as shellac.

5. A pharmaceutical agent according to claim 4 which comprises calcium acetate in the form of a capsule, tablet or pill coated with such a resin.

6. A pharmaceutical agent according to claim 5 which comprises between 0,1 grams and 100,0 grams of the acetate.

7. A pharmaceutical agent substantially as herein described and exemplified.

8. A method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, including the step of administering to the colon of a mammal an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof.

9. A method according to claim 8 wherein the agent is administered to the colon, via the digestive track of the mammal.

10. A method according to claim 8 or claim 9 wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

11. A method according to any one of claims 8 to 10 wherein the short chain fatty acid is acetic acid.

12. A method according to any one of claims 8 to 11 wherein the agent is administered in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

13. A method according to any one of claims 8 to 12 wherein the agent is administered to a human being in an amount of between 0,1 gram and 100,0 gram at least once a day.

14. A method for the treatment or prevention of conditions in mammals substantially as herein described and exemplified.

15. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal, in a method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity.

16. Use according to claim 15 of an agent wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

17. Use according to claim 15 or claim 16 of an agent wherein the short chain fatty acid is acetic acid.

18. Use according to any one of claims 15 to 17 of an agent wherein the protective coating comprises a natural or synthetic resin such as shellac.

19. Use according to any one of claims 15 to 18 of an agent wherein the agent is administered to a human being in an amount of between 0,1 gram and 100,0 gram at least once a day.

20. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in the manufacture of a medicament for use in a method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, the medicament having a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

21. Use according to claim 20 of an agent wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

22. Use according to claim 20 or claim 21 of an agent wherein the short chain fatty acid is acetic acid.

23. Use according to any one of claims 20 to 22 of an agent wherein the protective coating comprises a natural or synthetic resin such as shellac.

24. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof substantially as herein described and exemplified.

AMENDED CLAIMS

[received by the International Bureau on 2 February 1999 (02.02.99); original claim 12 cancelled; original claim 8 amended; claims 13-24 amended and renumbered as claims 12-23 other claims unchanged (6 pages)]

1. A pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, comprising a short chain fatty acid, or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

2. A pharmaceutical agent according to claim 1 wherein the pharmaceutically acceptable salt of the short chain fatty acid is the calcium salt thereof.

3. A pharmaceutical agent according to claim 1 or claim 2 wherein the short chain fatty acid comprises acetic acid.

4. A pharmaceutical agent according to any one of the preceding claims wherein the protective coating comprises a natural or synthetic resin such as shellac.

5. A pharmaceutical agent according to claim 4 which comprises calcium acetate in the form of a capsule, tablet or pill coated with such a resin.

6. A pharmaceutical agent according to claim 5 which comprises between 0,1 grams and 100,0 grams of the acetate.

7. A pharmaceutical agent substantially as herein described and exemplified.

8. A method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, including the step of administering to the colon of a mammal an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, the agent being administered in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

9. A method according to claim 8 wherein the agent is administered to the colon, via the digestive track of the mammal.

10. A method according to claim 8 or claim 9 wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

11. A method according to any one of claims 8 to 10 wherein the short chain fatty acid is acetic acid.

~~12. A method according to any one of claims 8 to 11 wherein the agent~~ is administered in a pharmaceutically acceptable ~~protective~~ coating which is resistant to digestion and ~~solution~~ in the stomach and small intestine of a ~~mammal~~, but digestible or soluble in the colon of a ~~mammal.~~

12. ~~13.~~ A method according to any one of claims 8 to ~~12~~ 11 wherein the agent is administered to a human being in an amount of between 0,1 gram and 100,0 gram at least once a day.

13. ~~14.~~ A method for the treatment or prevention of conditions in mammals substantially as herein described and exemplified.

14
~~15~~. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal, in a method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity.

15 14
~~16~~. Use according to claim ~~15~~ of an agent wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

16 14 15
~~17~~. Use according to claim ~~15~~ or claim 16 of an agent wherein the short chain fatty acid is acetic acid.

17 14 16
~~18~~. Use according to any one of claims ~~15~~ to ~~17~~ of an agent wherein the protective coating comprises a natural or synthetic resin such as shellac.

18 14 17
~~19~~. Use according to any one of claims ~~15~~ to ~~18~~ of an agent wherein the agent is administered to a human being in an amount of between 0,1 gram and 100,0 gram at least once a day.

19
~~20~~. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in the manufacture of a medicament for use in a method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, the medicament having a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

20 19
~~21~~. Use according to claim ~~20~~ of an agent wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

21 19 20
~~22~~. Use according to claim ~~20~~ or claim ~~21~~ of an agent wherein the short chain fatty acid is acetic acid.

22 19 21
23. Use according to any one of claims 20 to 22 of an agent wherein the protective coating comprises a natural or synthetic resin such as shellac.

23
24. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof substantially as herein described and exemplified.

INTERNATIONAL SEARCH REPORT

International Application No
PC EP 97/04875

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/19 A61K9/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 4 870 105 A (FORDTRAN JOHN S) 26 September 1989 see claims 1-8	1-7,14, 24 8-24

Y	PEAK ET AL.: "DNA damage produced by exposure of supercoiled plasmid DNA to high- and low LET ionizing radiation: effects of hydroxyl radical quenchers" INT. J. RADIAT. BIOL., vol. 67, no. 1, 1995, pages 1-6, XP002064519 see page 3, column 2, paragraph 3 - page 4, column 1, paragraph 1; figure 2; table 2	8-24

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 1998	3 December 1998 (03.12.98)

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	A. Jakobs

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GUARNIERI ET AL.: "Acetate Intolerance in Chronic Uremic Patients" NEPHRON, vol. 24, no. 4, 1979, pages 212-216, XP002064520 see abstract; figure 3 ---	8-24
Y	HAVERKATE ET AL.: "Influence of Some Organic and Inorganic Ions on Fibrinolysis in vitro" FOLIA HAEMATOL., vol. 103, no. 3, 1976, pages 389-398, XP002064521 see page 389, paragraph 1-3; tables 1,3 ---	8-24
Y	VREMAN ET AL.: "EFFECTS OF DIETARY ACETATE AND BICARBONATE ON EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS" ATHEROSCLEROSIS, vol. 35, no. 2, 1980, pages 145-153, XP002064522 see abstract; table 3 ---	8-24
Y	CHOHAN ET AL.: "Sodium Acetate Buffer: A diluent of Choice in the Clot Lysis Time Technique" THROMB. DIATH. HAEMORRH., vol. 33, no. 2, 1975, pages 226-229, XP002064523 see abstract; figure 1 ---	8-24
Y	CHOHAN ET AL.: "Fibrinolytic Activity at High Altitude and Sodium Acetate Buffer" THROMB. DIATH. HAEMORRH., vol. 32, no. 1, 1974, pages 65-70, XP002064524 see abstract; figure 1 ---	8-24
Y	TANDON ET AL.: "Synergistic Effects of Butyrate on Platelet Responses to Arachidonate, A23187, PGE1, and Forskolin" BLOOD, vol. 67, no. 2, 1986, pages 366-372, XP002064525 see abstract; figures 1,5; tables 1,2 --- -/--	8,15

INTERNATIONAL SEARCH REPORT

International Application No
P. /EP 97/04875

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MIRAGLIOTTA ET AL.: "Influence of short-chain fatty acids produced by anaerobic bacteria on procoagulant activity produced by Escherichia coli and Bacteroides fragilis-stimulated leucocytes: possible role in intra-abdominal abscess formation" MICROBIOS, vol. 75, no. 305, 1993, pages 233-240, XP002064526 see abstract; figures 1,2 ---	8-24
Y	GALLIMORE ET AL.: "Effect of diluents on blood clot lysis" J. CLIN. PATH., vol. 20, no. 3, 1967, pages 234-238, XP002064527 see figures 1-3; table 1 ---	8-24
X	US 4 721 716 A (NEESBY TORBEN E) 26 January 1988	1,2, 7-10, 12-16, 19-21,24
Y	see column 2, line 3 - line 60; claims 1,4 ---	4,18,23
X	WO 90 04334 A (ALPHA BETA TECHNOLOGY) 3 May 1990 see page 16, line 8 - line 19; claims 1,24 ---	1,8,9, 11,14, 15,19,20
Y	EP 0 616 802 A (TEIKOKU SEIYAKU KK) 28 September 1994 see claims 1,5 ---	4,18,23
E	NL 1 006 774 A (UNIV POTCHEFSTROOM (ZA)) 20 February 1998 see the whole document -----	1-24

4

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/EP 97/04875

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8-14 are directed to a method of treatment of the human/animal8 body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

In view of the large number of compounds, which are defined by the general definition(s)/formulae used in claims 1,2,4,7,8-10,12-16,18-21, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and / or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, chapter III, paragraph 2.3)